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TRANSMITTAL FORM

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Application Number	09/707,000
Filing Date	11/06/2000
First Named Inventor	Jon A. Wolff
Art Unit	1632
Examiner Name	Michael C. Wilson
Attorney Docket Number	Mirus.018.01

ENCLOSURES *(Check all that apply)*

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☐ Amendment/Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Reply to Missing Parts/ Incomplete Application
  - ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
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- ☐ Power of Attorney, Revocation Change of Correspondence Address
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- ☑ Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- ☐ Proprietary Information
- ☐ Status Letter
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Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	MARK K. JOHNSON		
Signature			
Printed name	MARK K. JOHNSON		
Date	June 29, 2006	Reg. No.	35,909

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	**09/707,000**
Applicants	:	**Jon A. Wolff, Vladimir Budker**
Filed	:	**11/06/2000**
Art Unit	:	**1632**
Examiner	:	**Wilson, Michael C.**
Docket No.	:	**Mirus.018.01**

For: **Intravascular Delivery of Nucleic Acid**

Commissioner of Patents
PO Box 1450
Alexandria, VA 2231-1450

AMENDED APPELLANT'S BRIEF under 37 CFR 1.192

(i) Real party in interest:

The real parties in interest are: Jon A. Wolff and Vladimir Budker and, by assignment, Mirus Corporation, which has changed its name to MirusBio Corporation under the laws of the State of Delaware and is located at 505 South Rosa Road, Madison, WI 53719.

(ii) Related appeals and interferences:

There are no interferences known to appellant, the appellant's legal representative, or assignee which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(iii) Status of Claims:

Claims 1-3, 6-9, 11-14, 16-22, 24-26, 28-31, 34-36, and 39 have been rejected and are hereby appealed.

Claims 4-5, 10, 15, 23, 27, 32, 33, 37, 38, and 40 have been canceled.

(iv) Status of amendments:

No Amendments have been filed subsequent to the final rejection.

(v) Summary of claimed subject matter:

The claimed subject matter is a process for delivering a polynucleotide to a cell in a mammalian limb. The inventors injected polynucleotides into Rhesus macaque monkey limb blood vessels, and limbs of other mammals, and caused the polynucleotide to be delivered to limb extravascular cells. Importantly, for both arm and leg injections, blood flow during the injection was impeded by a cuff (tourniquet) on the surface of the skin and/or fur, surrounding the arm or leg (Example 1 beginning on page 23, line 11; Example 3 beginning on page 25, line 15, and Example 10 beginning on page 32 line 9). This aspect is essential. Applying a cuff is non-invasive relative to prior art methods. According to the prior art, to clamp off a blood vessel, surgery must be performed and the claim applied while the surgical wound is open. In contrast, a cuff is simply wrapped around the limb and pressure applied, in the same way a sphygmomanometer cuff is applied to measure blood pressure.

A cuff surrounding the limb is defined in the specification as device placed external to the mammal's skin and which applies pressure against the limb to constrict blood vessels in an area underneath the cuff in amount sufficient to impede blood from flowing at a normal rate. Disclosed exemplary cuffs include a sphygmomanometer and a tourniquet, both of which are commonly used devises for occluding blood flow in a limb (page 5, lines 13-24).

Injection of a polynucleotide-containing solution into a limb blood vessel combined with occluding blood flow using the cuff around the limb results in increased permeability of blood vessels and delivery of polynucleotides to extravascular cells in the limb (page 2 lines 26-32; page 4 line 27 to page 25 line 3; and, page 17 line 8 to page 18 line 6). High levels of expression of delivered genes were found in muscle groups throughout the limb distal to the blood vessel occlusion (Table A, beginning on page 26, line 15; and, Table B, beginning on page 27). Expression levels in monkeys and rats were similar (Examples 7-10 beginning on page 30, line 19). Example 8 on page 31 is specific to expression of a therapeutic gene.

(vi) Grounds of rejection to be reviewed on appeal:

Whether claims 1-3, 6-9, 11-14, 16-22, 24-26, 28-31, 34-36, and 39 are unpatentable under 35 U.S.C. 112, first paragraph as failing to comply with the written description requirement.

Whether claims 1-3, 6-9, 11-14, 16-22, 24-26, 28-31, 34-36, and 39 are unpatentable under 35 U.S.C. 112, first paragraph not providing enablement.

Whether claims 1-3, 6-9, 11-14, 16-22, 24-26, 28-31, 34-36, and 39 are unpatentable under 35 U.S.C. 112, second paragraph for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

Whether claims 1, 3, 34-36 and 39 are unpatentable under 35 U.S.C. 102 as being anticipated by Milas *et al.* (Clin Cancer Res 1997).

Whether claims are unpatentable under the judicially created doctrine of obviousness-type double patenting:

- Claims 1-3, 6-9, 11-14, 16-22, 24-26, 28-31, 34-36, and 39 over copending Application No. 09/707,117;
- Claims 1-3, 37 and 39 over U.S. Patent, 6,379,966;
- Claims 1-3, 6-9, 11-14, 16-22, 24-26, 28-31, 34-36, and 39 over Application No. 09/917,154 (now abandoned).

(vii) Argument:

Rejection of the Claims under 35 U.S.C. 112, first paragraph.

(A) Response to written description rejection:

The initial rejection on page 4 of the Office Action states that the phrase "syringe needle" in claims 1 and 39 does not have support in the specification. Support for the term "needle" is found in the specification in example 8 on page 31. Injecting a fluid into a vessel is described throughout the specification. Applicants believe that it is readily apparent to a person skilled in the art that a needle used to inject a solution into a vessel or a mammal would be a syringe needle. To support their contention, the common term "syringe" is defined in the Merriam-Webster Dictionary as: **1.** a device used to inject fluids into or withdraw them from something (as the body or its cavities): an instrument (as for the injection of medicine or the withdrawal of bodily

fluids) that consists of a hollow barrel fitted with a plunger and a hollow needle. As an alternative to the term "syringe needle" Applicants clearly have support for the broad term "needle" and the use of the term "syringe" narrows the term needle by the common English definition of the word. Therefore, Applicants submit that there is support in the Specification for the words "syringe needle."

The second rejection on page 4 states that the phrase "impeding blood flow to the surface of the skin" in claims 1 and 39 does not have support in the specification and is new matter. The phrase written in the Office Action is a fragment of the entire phrase as stated in the claims and is not correctly presented. Claims 1 and 39 actually state "applying a device for impeding blood flow to the surface of the skin of said limb". The device is a device for impeding blood flow and it is applied to the surface of the skin. Support for such a device is found in the specification on page 3 lines 8-24, and page 5 lines 5-24. A sphygmomanometer and a tourniquet are provided as two exemplary cuffs (or devices for impeding blood flow), both of which are readily envisioned by persons skilled in the art. Applicants contend that the phrase has support in the specification and should not be considered new matter.

The rejection states that the phrase "sufficient pressure" in claim 1 does not have support in the specification. The full text of the claim states "applying sufficient pressure against said limb with said device to occlude blood flow to said limb". Applying pressure to occlude blood flow is supported in the specification on page 3 lines 1-11 and lines 21-24, page 5 lines 6-11, page 23 lines 22-24, and page 33 lines 4-5. The term "sufficient" is a plain language term that is defined in every English dictionary. As explained in the Specification, blood flow to the limb must be impeded for the material being delivered to escape the vessel to the extravascular space. One can imagine a cuff placed around a limb without sufficient pressure to impede the blood flow. The term "sufficient" is simply a common English term to clarify that enough pressure must be applied against a limb to impede or occlude the blood running through the limb. Therefore, Applicants believe that the phrase "sufficient pressure" is supported by the Specification.

(B) Response to enablement rejection:

In paragraph II. on page 5, the rejection under 35 U.S.C. 112 states that the Specification is not enabling for: "1) injecting the polynucleotide to the limb proximally to the applied pressure and obtaining delivery of the polynucleotide to the skeletal muscle cells of the limb distally to the applied pressure."

Claim 1 states in step (d) "injecting a solution containing the polynucleotides through said injector into the lumen of said artery distal to the occlusion thereby delivering the polynucleotides to said skeletal muscle cells distal to said occlusion in the limb." (underline added). Claim 39 similarly states that the solution is injected "distal to the occlusion".

It appears that the Examiner uses the words "proximally to the applied pressure" as the point where the nucleic acid is injected to bolster his statement that the claim is not enabled by the Specification. However, Applicants teach injecting nucleic acids distal to the applied pressure where they are also delivered (page 23 lines 22-23, page 32 lines 11-12). Therefore, Applicants do not disagree with the Examiner's statement but are confused by its relevance.

In the Action, beginning in the first line on page 6, it states: "claim 1 encompasses inserting the injector to the limb proximally to the applied pressure and injecting the polynucleotides distally to the applied pressure." This is true and is one of the methods used in the Examples. For instance, a catheter was inserted into a vessel proximal to the applied pressure. The catheter was then pushed through the vessel beyond the position of the applied pressure to the distal side where the nucleic acids were injected and delivered. (The biological point of reference to describe distal and proximal is the center of the body or heart.)

In the first full paragraph on page 6 of the Action it states: "claim 3 encompasses injecting a viral vector into a blood vessel of a limb to obtain delivery to a skeletal muscle cell." This statement is accurate and is supported by the Specification on page 15 lines 13-25.

In the second paragraph on page 6, the Action states: "claims 6-9, 11-14, 16-22, 24-26, 28, 31 and 34-36 require delivery to specific muscles within the limbs." This

statement is accurate and each Example in the Specification shows delivery to limb muscle. The delivery in each instance is shown to be distal to the applied pressure, in the limb muscle, with delivery extending to the most extreme muscles of the limb such as muscle in feet and hands. Again, it is unclear why the Examiner's statement is put forth.

The 3rd paragraph on page 6 states that claim 39 "encompasses inserting the injector to the limb proximally to the applied pressure and injecting the polynucleotides distally to the applied pressure." As stated earlier, this statement is correct. Claim 39 is not limited to inserting the injector distally to the applied pressure. A method of injection includes inserting a catheter into a blood vessel proximal to the applied pressure for injection and delivery distal to the applied pressure.

In the last paragraph on page 6 the Action states that vector targeting is unpredictable and inefficient according to some prior art references. Applicants agree with that statement since a novel aspect of their invention is derived from their ability to deliver nucleic acids to a "desired tissue" such as limb muscles. This novelty has been demonstrated in each of the Examples written in the Specification. Applicants believe that they have significantly added to the art published in 1995 -1998 as listed by the Examiner.

On page 7 of the Office Action, first and second paragraphs, it states that "the specification does not enable delivering the polynucleotide to a blood vessel of a limb proximal to the applied pressure and delivering the polynucleotide to skeletal muscle cells of the limb distal to the applied pressure as broadly encompassed by claims 1 and 39." This statement is confusing in that it lists delivery proximal and distal at the same time. Applicants clearly have not supported that statement and have not claimed it. As stated earlier, Applicants claim injection and delivery into an artery distal to the applied pressure (occlusion). Paradoxically, the Action states in the last sentence of this paragraph that "claims 1 and 39 should be limited to injecting the polynucleotide distal to the site of applied pressure."

In the last paragraph on page 7 and continuing on page 8 the Action states that the specification does not discuss how to overcome the problems described by the prior

art Milas. Milas taught perfusing a limb by inserting a solution containing adenovirus into an artery while allowing brisk outflow through a vein. Milas notes that providing brisk outflow is important (ref. page 2202, column 1).

In contrast, Applicant's invention teaches prevention of outflow. This difference is one reason why Milas failed to observe delivery to skeletal muscle cells in the limb. Applicants' method provides for delivery of nucleic acid to skeletal muscle cells in the limb by utilizing a tourniquet around the limb without requiring surgery. Applicants specifically teach that not occluding blood flow results in no delivery (page 30 lines 23-24). Note that the method of Milas requires perfusion of the limb and permanent occlusion of vessels (page 2198, column 2, last paragraph). The method described by Milas relies on collateral blood flow to supply to limb following the procedure (page 2199, first paragraph, last full paragraph). Applicants' method does not recommend either of these procedures.

In the Action on page 8, beginning on line 7, it states: "The specification does not discuss how to overcome the problems described by Milas." Applicants' Specification teaches delivery of nucleic acids to muscle cells in limbs. The Milas reference is directed at delivering DNA to tumors and specifically states that it did not deliver to muscle tissue (Ref. page 2201, column 2, line 16). Methods of delivery to limb muscle are not required to overcome the problems of prior art directed to delivery of DNA to tumor cells.

In the last paragraph on page 8 of the Action it states that the specification does not enable delivering any polynucleotide as broadly claimed. The Examiner states that the specification is enabling for delivering a naked DNA encoding a protein operably linked to a promoter. However, it is known in the art that either the presence or absence of a promoter sequence in the polynucleotide is not likely to have an effect on whether or not the polynucleotide is delivered by the method described in the Specification. For example, Applicants have shown delivery of polynucleotides, not having a promoter sequence, complexed with a polymer (U.S. Patent No. 6,740,336) as well as delivery of small interfering RNA polynucleotides (U.S. Serial No. 10/012,804) representing a range of polynucleotides which have been delivered without using an exterior tourniquet or cuff. It would be unreasonable to infer that

when using a tourniquet or cuff, a promoter sequence is necessary for polynucleotide delivery.

On page 9, first paragraph, the action states that "For the delivery to have an enabled use, it must encode a protein that is expressed to detectable levels in the cell." However, it is widely known in the art that antisense polynucleotides, and the more recently discovered small interfering RNA, can be used to block expression of cellularly expressed genes. Inhibition of gene expression is a widely accepted use and such polynucleotides are typically small oligonucleotides that are neither linked to a promoter nor encode a protein. (See page 6 lines 18-24, page 14 lines 14-30, and page 14 line 32 to page 15 line 2. Therefore, expression is not a required step for successful delivery of a polynucleotide into a cell.

Further on page 9, Applicants' examples are used to limit the claims. The Action states that since the examples describe only genes linked to promoters, the claims should also be limited. However, as discussed in the prior paragraph, inhibition of gene expression is a well known use of delivery methods. Applicants' described methods can be used to deliver either polynucleotides that are expressible or polynucleotides that inhibit expression equally well and should not be unreasonably limited to one or the other. The more important factor is the non-invasiveness of Applicants' procedure.

On page 10, first paragraph, the Action states that "RNA not translated into protein would not be 'expressed in the skeletal muscle cell' as claimed." However, Applicants have not claimed expression. Therefore, Applicants cannot formulate a response to the statement.

In the same paragraph: "The specification does not describe the polynucleotide recited in claims 1 or 39 as encompassing an RNA molecule that is not translated into protein 'but has a cellular function itself.' Therefore, the claims should be limited to DNA encoding a protein operably linked to a promoter." However, as discussed in previous paragraphs, the Specification does indeed describe expression inhibitors, including RNA, on page 6 lines 18-24, page 14 lines 14-30, and page 14 line 32 to page 15 line 2.

The action states on page 10, second paragraph, that the specification has not overcome the unpredictability of targeting polynucleotides to the desired tissue as established by Miller (1995), Deonarain (1998), Verna (1997) and Crystal (1995). Applicants respectfully remind the Examiner that each of these references was published prior to the filing date of Applicants' Specification and none of them contemplates the method described by Applicants. Prior art that makes an attempt to target polynucleotides to desired tissues cannot be used to refute subsequent art that effectuates targeted delivery of polynucleotides. Applicants have described and demonstrated targeted delivery to limb muscle. Therefore, the cited prior art, which couldn't target tissues, can no longer be considered the "established" methods.

Rejection of the claims under 35 U.S.C. 112, second paragraph (Office Action page 10):

The Examiner states that the phrase "to occlude blood vessels in the limb" in claim 1 is an intended use and may not occur. Applicants cannot respond because the quoted phrase is not recited in claim 1. The claim provides for applying a device for impeding blood flow to the limb (step b), and applying pressure against the limb with the device to occlude blood flow to the limb (step c).

In the second paragraph on page 11, the Examiner states that the phrase "impeding blood flow to the surface of the skin" is unclear. The Examiner has taken the phrase out of context thus misinterpreting of the phrase. The complete phrase is: "applying a device for impeding blood flow to the surface of the skin of said limb." It is taken directly from the specification (page 5 line 13). That the device is applied to the surface of the skin is described in the specification on page 5 lines 14-15.

The Action states that the phrase "sufficient pressure" required to "occlude blood flow to said limb" is unclear because the specification and the art, at the time of filing, do not define the amount of pressure required to occlude blood flow. Applicants disagree.

The specification provides sphygmomanometers and tourniquets as examples of devices to occlude blood flow. Sphygmomanometers and tourniquets are widely used

devices which, as part of their normal use, apply pressure against a limb to occlude blood flow. The specification further describes how these devices occlude blood blow to a limb; see page 5 lines 15-19.

It is well known that an individual's blood pressure varies from person to person. It follows that variable applications of pressure would be required to occlude blood flow in different people. People trained in measuring blood pressure can easily determine when blood flow has been occluded in any individual simply by listening through a stethoscope. Simple mechanical/electrical devices that can also detect occlusion of blood flow in a particular individual have been commercially available for many years. On page 5, lines 17-19, the Specification states: "The vessel walls are forced to constrict in an area underneath the cuff in amount sufficient to impede blood from flowing at a normal rate." Since the amount of pressure required to occlude blood flow varies from person to person, and the amount of pressure required to accomplish such occlusion is easily determined, Applicants believe that the terminology "sufficient pressure" is clear and does not require further definition.

The Action states that the phrase "said occlusion" in claim 1 step d lack antecedent basis. Applicants respectfully disagree. Claim 1 step c states "to occlude blood flow". If blood flow is occluded, an occlusion is formed. The Merriam-Webster dictionary defines occlusion as **1**: the act of occluding **:** the state of being occluded. Dictionary.com further adds **2**: The act of occluding or the state of being occluded. Therefore, Applicants believe that the terminology "said occlusion" is used properly.

On page 12, first paragraph, the action states that the metes and bound of "cuff" (claims 35 and 36) are unclear. The action states that while a sphygmomanometer cuff can be envisioned, other cuffs can not be envisioned and that it can not be determined whether a cuff is a "device for impeding blood flow through mammalian internal blood vessels" (page 5 line 13-14 of the specification) or a "device applied exterior to the mammal's skin and touches the skin in a non-invasive manner" (page 5 lines 14-15 of the specification).

Applicants point to their Specification on page 5, lines 14-15, where it specifically states "However, for purposes of the claims, cuff refers specifically to a device

applied exterior to the mammal's skin and touches the skin in a non-invasive manner." Applicants further note that a cuff applied exterior to the mammal's skin, when applied according to the specification, will impede blood flow through mammalian internal blood vessels (page 5 lines 15-19).

In the last paragraph on page 12 of the Action, claim 39 has been rejected because "mere delivery of polynucleotides to cells does not have a disclosed use." Applicants disagree and have addressed this issue in previous paragraphs in response to rejections under 35 U.S.C. 112, first paragraph. Applicants further note that delivery of polynucleotides to cells in vivo has use to one having ordinary skill in the art. A search of the scientific literature for gene therapy alone will net thousands of articles describing research, benefits and effects of delivering polynucleotides to cell in vivo. Further, the specification outlines a number of potential uses: altering the endogenous properties of the cell (page 2 lines 29-30), block gene expression (page 6 line 18), cleave cellular RNA (page 6 lines 21-22), block transcription (page 6 line 22), bind to cellular proteins (page 6 line 23), expressing proteins (page 6 lines 28-29), therapeutic effects (page 7 line 28 to page 8 line 14). All of these are legitimate commercial uses.

In the Action on page 10, first paragraph, claim 39, step c has been determined to be indefinite because it is not "an active step." Applicants do not understand this statement which makes it difficult to formulate a response. Nevertheless, the ability to deliver polynucleotides in a non-invasive manner, of which retaining complete limb function after the procedure being highly significant, is one of the more novel aspects of the invention. Inclusion of this aspect into the claims is deemed to be important by Applicants.

Rejection of the claims under 35 U.S.C. 102:

Claims 1,3 34-35 and 39 have been rejected under 35 U.S.C. 102(b) as being anticipated by Milas (1997). The Examiner states that the method of Milas inherently obtains delivery of polynucleotides to skeletal muscles because the muscles showed small inflammatory cell infiltrates. It is the Examiner's contention that the presence of small inflammatory cell infiltrates indicate the presence of foreign material. The Examiner also states that Fig. 3 on page 2200 indicates that red blood cells leaked

throughout the limb, which implies that the adenovirus also leaked throughout the limb. These interpretations reflect a misunderstanding of biological processes.

An inflammatory cell infiltrate merely indicates the presence of immune cells in the region. Adenovirus ("foreign material") may be present in the leg using the Milas method. However, it is Applicants' contention that the foreign substance is not delivered to skeletal muscle cells in the leg. As pointed out in previous replies, this contention is supported in the Milas et al. reference. Milas et al. state, on page 2201, second column, first full paragraph, that "no β-gal staining was apparent in the muscular tissues of the perfused leg." Milas et al. further state, in the same paragraph, that the only detectable difference between the purfused and nonperfused leg was the presence of inflammatory cell infiltrates.

The Examiner states that Fig. 3 in the Milas et al. reference shows that red blood cells leaked throughout the limb. That statement is not accurate. Milas et al. provide no indication of red blood cells 'leaking' out of the vasculature. Fig. 3 and the corresponding evidence are presented by Milas et al. solely to demonstrate the amount of systemic leakage of blood out of the isolated leg and into the rest of the rat body during the procedure. The Milas et al. reference states on page 2201, column 1, first full paragraph, in regard to Fig. 3: "it was pertinent to determine the effectiveness of limb isolation from the systemic circulation." "The predominant concentration of the leaked perfusate was in the liver and portal system." The experiment does not describe delivery of any material, including red blood cells, out of the vascular system.

The Action states that the method of Milas et al. "most definitely occludes inflow and outflow of blood to the leg." That statement is inaccurate. Milas et al. clearly provide for inflow of fluid into the leg, specifically at a rate of 2.4 ml per minute (page 2199, column 1). Milas et al. also clearly provide for fluid flow out of the leg: "the perfusion pump was positioned below the plane of the animal to allow venous outflow" (page 2199 column 1); "the perfusate entered via the fermoral artery and returned via the femoral vein" (page 2199 column 1); "cannulation of the femoral vein with resultant brisk outflow is critical for the success of the procedure" (2202, paragraph 1). Applicants' claims do not contemplate an invasive perfusion technique as put forth in

Milas et al. Therefore, Applicants believe that the Milas et al. reference is not a proper §102 reference against their claims.

Obviousness-type double patenting:

On page 14, section V., claims 1-3, 6-9, 11-14, 16-22, 24-26, 28-31, 33-36, and 39 are provisionally rejected for obviousness-type double patenting when compared to claims 1-42 of copending Application No. 09/707,117.

However, the prior art and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person. Therefore, the provisional rejection is obviated.

In the Office Action on page 14, the last paragraph, claims 1-3, 37 and 39 are rejected for double patenting over claim 1 of U.S. Patent No. 6,379,966. Claim 1 in the '966 patent reads as follows:

> 1. A process for delivering a polynucleotide complexed with a compound into an extravascular parenchymal cell of a mammal, comprising:
> a) mixing the polynucleotide and a polymer to form a complex wherein the complex has a zeta potential which is not positive;
> b) inserting the polynucleotide into a mammalian blood vessel, in vivo;
> c) increasing permeability of the blood vessel;
> d) passing the complex through the blood vessel;
> e) delivering the complex into the mammalian extravascular parenchymal cell; and
> f) expressing the polynucleotide.

The Action states that the claims of the '966 and Applicants' claims are not patentably distinct because the method of '966 is an obvious species of Applicants' claims.

In contrast to the '966 claim 1, Applicants have described non-invasive methods for delivering polynucleotides to skeletal muscle cells in a mammalian limb by applying a device for impeding blood flow to the surface of the skin of a limb (exterior occlusion). The '966 claim 1 is not a species of Applicants' claims because the '966 patent and claims do not require occlusion. Delivery in the '966 is accomplished by hydrodynamic delivery methods where impeding blood flow (occlusion) is not necessary. Therefore, the '966 patent claims cannot be a species of Applicants claims and the two sets of claims are patentably distinct.

In section VII, claims1-3, 6-9, 11-14, 16-22, 24-26, 28-31, 33-36, and 39 are provisionally rejected for obviousness-type double patenting when compared to claims 1-42 of copending Application No. 09/917,154.

However Application No. 09/917,154 has been abandoned which obviates the provisional rejection.

(viii) Claims Appendix:

1. (previously presented) An *in vivo* process for delivering polynucleotides to skeletal muscle cells in a limb of a mammal, comprising:
   a) inserting an injector selected from the group consisting of a syringe needle and catheter into an artery in said limb;
   b) applying a device for impeding blood flow to the surface of the skin of said limb;
   c) applying sufficient pressure against said limb with said device to occlude blood flow to said limb; and,
   d) injecting a solution containing the polynucleotides through said injector into the lumen of said artery distal to the occlusion thereby delivering the polynucleotides to said skeletal muscle cells distal to said occlusion in the limb.
2. (original) The process of claim 1 wherein the polynucleotide consists of naked DNA.
3. (original) The process of claim 1 wherein the polynucleotide is selected from the group consisting of a viral vector and a non-viral vector.

4-5. (cancelled)

6. (previously presented) The process of claim 1 wherein the muscle cell consists of a leg muscle cell.
7. (previously presented) The process of claim 1 wherein the muscle cell consists of an arm muscle cell.
8. (original) The process of claim 7 wherein the arm muscle cell consists of an anterior muscle cell.
9. (original) The process of claim 8 wherein the anterior muscle cell consists of an anterior superficial muscle cell.
10. (canceled)
11. (previously presented) The process of claim 9 wherein the muscle cell is selected from the group consisting of palmaris longus, pronator teres, flexor carpi radialis, flexor carpi ulnaris, and flexor digitorum superficialis.
12. (previously presented) The process of claim 8 wherein the anterior muscle cell is selected from the group consisting of flexor digitorum profundus, and pronator quadratus.

13. (original) The process of claim 7 wherein the arm muscle cell consists of a posterior muscle cell.
14. (original) The process of claim 13 wherein the posterior muscle cell consists of a posterior superficial muscle cell.
15. (canceled)
16. (previously presented) The process of claim 14 wherein the muscle cell is selected from the group consisting of brachioradialis, extensor carpi radialis longus, extensor carpi, radialis brevis, extensor digitorum, anconeus, extensor and carpi ulnaris.
17. (previously presented) The process of claim 13 wherein the posterior muscle cell is selected from the group consisting of supinator, extensor pollicis longus, abductor pollicis longus, extensor digiti secund et teriti, and extensor digiti quart et minimi.
18. (original) The process of claim 7 wherein the arm muscle cell consists of a hand muscle cell.
19. (original) The process of claim 18 wherein the hand muscle cell consists of a thumb muscle cell.
20. (original) The process of claim 18 wherein the hand muscle cell consists of an interosseus cell.
21. (original) The process of claim 6 wherein the leg muscle cell consists of a posterior muscle cell.
22. (original) The process of claim 21 wherein the posterior muscle cell consists of a superficial cell.
23. (canceled)
24. (original) The process of claim 22 wherein the superficial cell is selected from the group consisting of gastrocnemius and soleus.
25. (previously presented) The process of claim 21 wherein the posterior muscle cell is selected from the group consisting of popliteus, flexor digitorum longus, flexor hallucis longus, and tibialis posterior.
26. (original) The process of claim 6 wherein the leg muscle cell consists of a anterior muscle cell.
27. (canceled)
28. (original) The process of claim 6 wherein the leg muscle cell consists of a foot muscle cell.

29. (original) The process of claim 26 wherein the anterior muscle cell is selected from the group consisting of tibialis anterior, extensor hallucis longus, extensor digitorum longus, and abductor hallucis longus.

30. (previously presented) The process of claim 6 wherein the leg muscle cell is selected from the group consisting of peronaus longus and peronaus brevis.

31. (original) The process of claim 28 wherein the foot muscle cell is selected from the group consisting of extensor digitorum brevis and extensor hallucis brevis.

32-33. (canceled)

34. (previously presented) The process of claim 33 wherein said device for impeding blood flow consists of a tourniquet .

35. (previously presented) The process of claim 33 wherein said device for impeding blood flow consists of a cuff surrounding said limb.

36. (previously presented) The process of claim 35 wherein said device for impeding blood flow consists of a sphygmomanometer cuff.

37.-38. (canceled)

39) (previously presented) An *in vivo* process for delivering polynucleotides to skeletal muscle cells in a limb of a mammal, comprising:

a) inserting an injector selected from the group consisting of syringe needle and catheter into a blood vessel in said limb in the mammal and applying pressure to the blood vessel wherein the pressure occludes blood flow through said blood vessel and is applied to the skin of said limb by a device external to the skin of said mammal and;

b) injecting a solution containing the polynucleotides into the lumen of said blood vessel distal to the occlusion thereby delivering the polynucleotides to said skeletal muscle cells in the limb distal to the occlusion; and,

c) wherein function of the limb is not affected by inserting the injector, applying pressure to the vessel, and injecting the solution.

40. (canceled)

(ix) Evidence appendix:

The §1.132 Declaration was apparently entered into the record when the examiner drafted the August 19, 2004 Office Action, however, it was not discussed in that Action.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: **Jon A. Wolff,**
Vladimir Budker

Serial No.: **09/707,000**

Filed: **11/06/2000**

Group Art Unit: **1632**

Examiner: **Michael C. Wilson**

For: **Intravascular Delivery of Nucleic Acid**

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
PO Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

I, Jon A. Wolff, hereby declare as follows:

1. I am an inventor of the captioned application.
2. I submit with this Declaration and Response further experimental material (attached) illustrating: delivery of polynucleotide encoding therapeutic proteins, VEGF and EPO, to limb skeletal muscle cells. The experiments were performed according to the methods provided in the Specification.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

_____

Jon A. Wolff Date

Increased vascularization following delivery of a therapeutic polynucleotide to primate limb. DNA delivery was performed via brachial artery with blood flow blocked by a sphygmomanometer cuff proximately to the injection site. Left arm was transfected with VEGF, while right arm was transfected with EPO. The *Sartorious musle* from left leg was used as non-injected control. A male Rhesus monkey weighing 14 kg was used for these injections. The animal was anesthetized with Ketamin (10-15 mg/kg). A modified pediatric blood pressure cuff was positioned on the upper arm. The brachial artery was cannulated with a 4 F angiography catheter. The catheter was advanced so that the tip was positioned just below the blood pressure cuff. Prior to the injection, the blood pressure cuff was inflated so that the cuff pressure was at least 20 mmHg higher than the systolic blood pressure. After cuff inflation, papaverine (5mg in 30 ml of saline) was injected by hand (~8 to 10 seconds). After 5 min, the pDNA solution was delivered rapidly with a high volume injection system. For the EPO injection, 10 mg of pDNA was added to 170 ml of saline and injected at a rate of 6.8 ml per second. For the VEGF injection, 10 mg of pDNA was added to 150 ml of saline, and injected at a rate of 5.4 ml per second.

After 65 days, the animal was euthanized by overdose I.V. injection of pentobarbital Ketamin (10 mg/kg). The entire *Pronator quadratus* and *Pronator teres* muscles from both sides were immediately harvested and fixed for 3 day in 10% neutral buffered formalin (VWR, Cleveland, OH). After fixation, an identical grossing was performed for left and right muscles and slices across the longitudinal muscles were taken. Specimens were routinely processed and embedded into paraffin (Sherwood Medical, St. Louis, MO). Four microns sections were mounted onto precleaned slides, and stained with hematoxylin and eosin (Surgipath, Richmond, IL) for pathological evaluation. Sections were examined under Axioplan-2 microscope and pictures were taken with the aid of AxioCam digital camera (both from Carl Zeiss, Goettingen, Germany).

To evaluate the effect of VEGF plasmid delivery on cell composition in muscle tissue and neo-angiogenesis, we used monoclonal mouse anti-human CD31 antibody (DAKO Corporation, Carpinteria CA). The immunostaining was performed using a standard protocol for paraffin sections. Briefly: four microns paraffin sections were deparaffinized and re-hydrated. Antigen retrieval was performed with DAKO Target

Retrieval Solution (DAKO Corporation, Carpinteria CA) for 20 min at 97°C. To reduce non-specific binding the section were incubated in PBS containing 1% (wt/vol) BSA for 20 min at RT. Primary antibody 1:30 in PBS/BSA were applied for 30 min at RT. CD31 antibody were visualized with donkey anti-mouse Cy3-conjugated IgG, 1:400 (Jackson Immunoresearch Lab, West Grove PA) for 1 h at RT. ToPro-3 (Molecular Probes Inc.) was used for nuclei staining; 1:70,000 dilution incubated for 15 min at RT. Sections were mounted with Vectashield non-fluorescent mounting medium and examined under confocal Zeiss LSM 510 microscope (Carl Zeiss, Goettingen, Germany). Images were collected randomly under 400× magnification, each image representing 0.106 sq mm. Because muscle fibers and red blood cells have an autofluorescence in FITC channel we use 488 nm laser to visualize these structures.

Morphometry analysis. Coded mages were opened in Adobe Photoshop 5.5 having image size 7 × 7 inches in 1 × 7 inches window, and a grid with rulers was overlaid. The number of muscle fibers, CD31 positive cells and total nuclei was counted in all 7 image's strips consecutively, without any knowledge of experimental design. T-Test for Two-Sample Unequal Variances was used for statistical analysis.

Results: Microscopic evaluation did not reveal any notable pathology in either muscle regardless of the gene delivered. Also, neither muscle showed any notable presence of inflammatory cells, except of few macrophages. Necrosis of single muscle fibers was extremely rare in both, occupying negligible volume and was not associated with infiltration/vascularization. However, in muscles transfected with VEGF-165 plasmid, the interstitial cell and vascular density (observed in H&E-stained slides) was obviously increased (FIG. 4), as compare to EPO plasmid administered muscle (FIG. 4). Based on morphologic evaluation, these newly arrived interstitial cells we suggested to be endothelial and adventitial cells, smooth muscle cells, and fibroblasts. To evaluate participation of endothelial cells in this neo-morphogenesis, we have counted the number of CD31 positive cells in EPO and VEGF delivered *Pronator quadratus* muscles (FIG. 5). To assure that comparable specimens were analyzed in right and left muscles, the number of muscle fibers was counted per area unit (0.106 sq mm). The VEGF and EPO administered muscles were not different in muscle fiber

number (means 30.5 and 31.6). The number of CD31 positive cells however was significantly increased by 61.7% $p<0.001$ (means 53.2 vs 32.9).

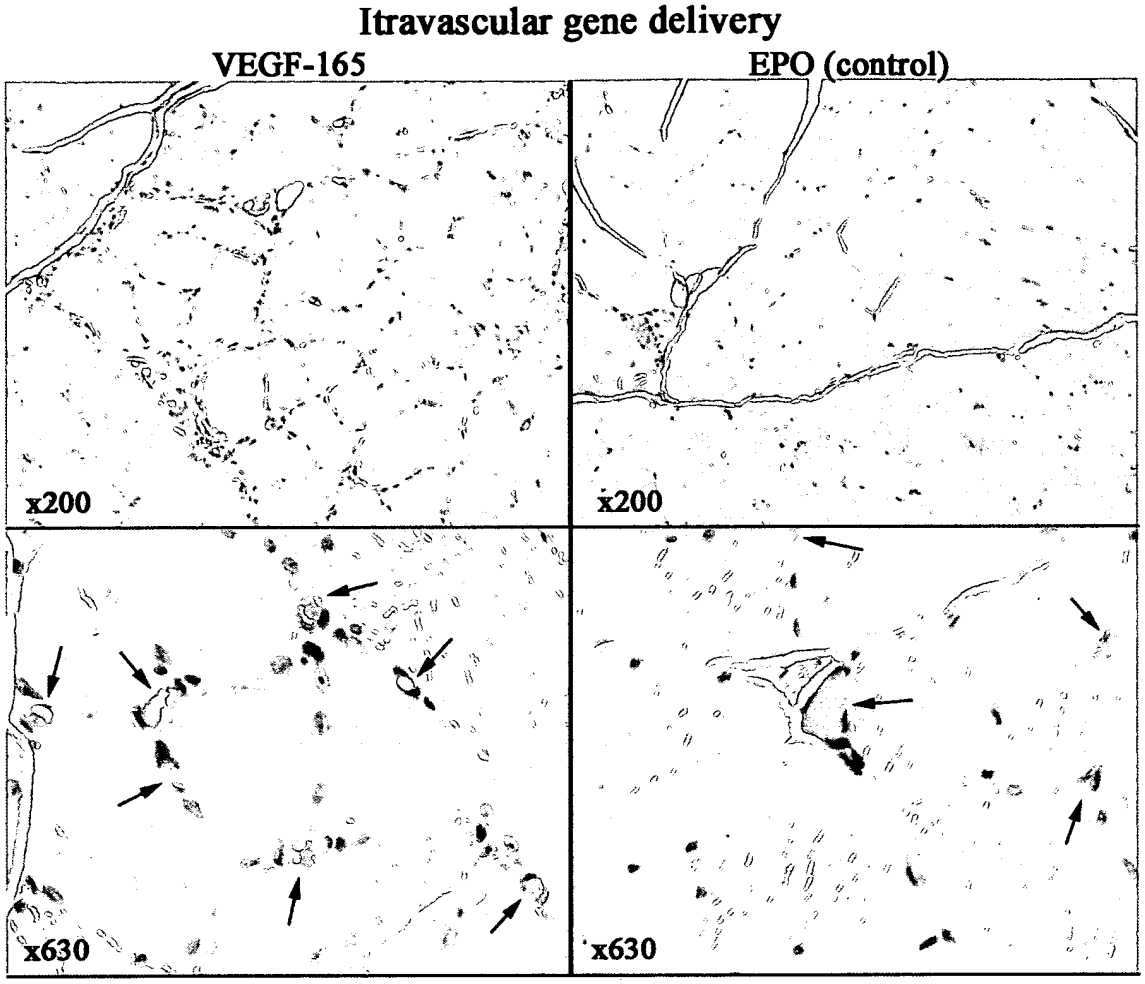

FIG. 4

Immunofluorescent staining (red) for endothelial cells (CD31)

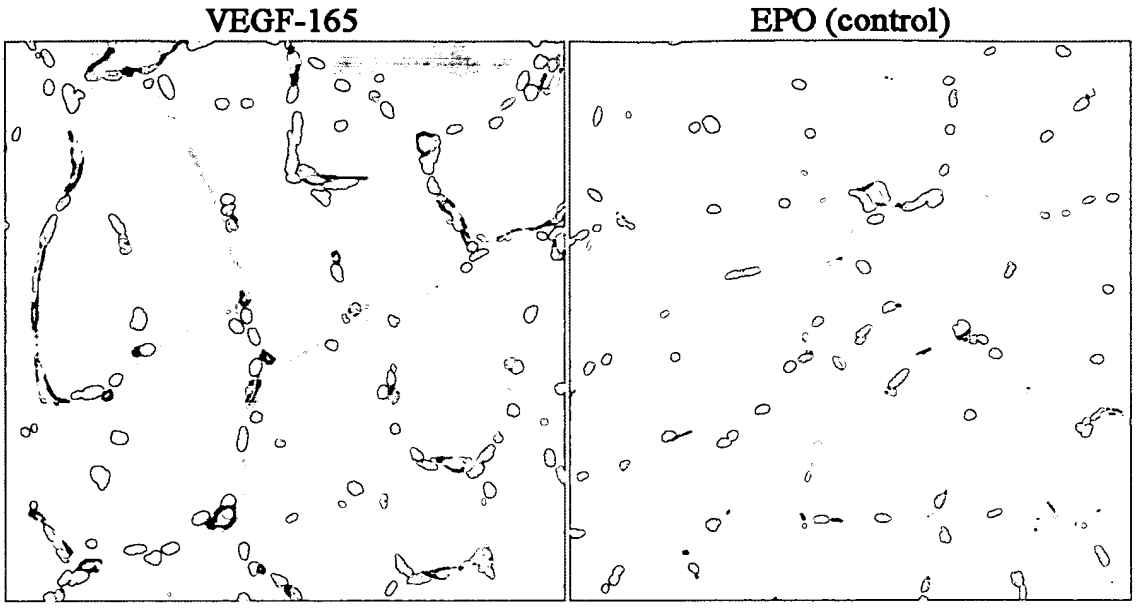

FIG. 5

(x) Related proceedings appendix: None

Pages 1-24 are
Respectfully submitted,

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I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as express mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on this date: June 29, 2006.

Mark K Johnson